Eric F. Bernstein, M.D.
504 Lippincott Drive
Marlton, NJ 08053
856-797-9099 phone
856-797-0277 fax

January 20, 2003

Kenato George, Esq.
U.S. Patent Examiner
USPTO
19111 south Clark St., 7th Floor
Arlington, VA

Dear Mr. George:

This letter concerns the patent application, *Use of serine protease inhibitors in the prevention of photoaging.* This idea was conceived quite sometime ago and was documented by Yann Echelard of Genzyme Transgenics Corporation in his laboratory notebook. Subsequent to developing this idea was tested in a well-established and patented in-vitro model of cutaneous photoaging.

The photoaging model is a patented rapid system to model skin photoaging and test compounds that may prevent photoaging (patents: *An in vivo and in vitro model of cutaneous photoaging* Patent 5,648,061, Issued July 15, 1997 and 6,018,098, Issued January 25, 2000). Compounds that prevent photoaging have been patented based solely on data from this system. Specifically the nitroxide, tempol, that protects against photoaging by blocking free radicals has been patented as an agent that prevents photoaging solely using data from our model (*Use of Tempol in the prevention of photoaging*, Patent 5,840,734, Issued November 24, 1998). Serine protease inhibitors work by blocking changes in the elastin gene. Since our model is based on the elastin gene, it is even better suited to measure the ability of serine protease inhibitors to prevent photoaging than any other type of compounds.

A discussion of photoaging and how are model relates follows below. The main alteration that occurs in skin that is aged from the sun is the accumulation of massive amounts of abnormal elastic tissue, and this elastic tissue is called 'solar elastosis'. As apposed to the normal elastin in skin that allows the skin to stretch and recoil, solar elastosis replaces the normal collagen-rich skin with its stringy elastic fibers with abnormal clumps of non-functional elastic material. This material takes up space in the skin and replaces the normal functioning skin. Thus sun-damaged skin appears wrinkly, and saggy.

Normal Skin (elastin black/collagen red) Sun-damaged skin (elastin black/collagen red)

To model this skin photoaging in an animal system requires many months. As with humans, skin aging requires almost half a lifetime in a mouse. To excelerate this aging process, we utilized a mouse containing the human elastin promoter with a chloramphenicol acetyl-transferase (CAT) construct. Skin and cells from these mice will demonstrate a rapid increase in elasin promoter activity, which is the first event that occurs in cuteaneous photoaging in people. Thus these mice and their cells provide a very sensitive model of skin photoaging. Instead of waiting half a mouse's lifetime for the elastin to accumulate as it does in a person, we measure the first event that occurs during the process of forming solar elastosis. We do this in a mouse or cell system using the *human* elastin promoter. We have shown in humans that the first event in elastin deposition in photodamaged skin is elastin promoter activation. We have tested numerous compounds in this system including sunscreens, antioxidants and the serine protease inhibitor alpha-1-antitrypsin, and supplied the laboratory notebooks from these experiments as evidence. Thus the idea to use serine protease inhibitors as an antiaging compound as well as the testing to demonstrate their effectiveness have been performed before the filing of the Lezdey patent.

References pertaining to the photoaging model and its use are printed below and copies have been included for your files:

1. Bernstein EF, Chen QY, Tamai K, Resnik KS, Li K, Uitto J. Enhanced elastin and fibrillin gene expression in chronically photodamaged skin. J Invest Dermatol. 103:182-186, 1994.
2. Bernstein EF, Brown DB, Urbach F, Forbes D, Del Monaco M, Wu M, Katchman SD, Uitto J. Ultraviolet radiation activates the human elastin promoter in transgenic mice: A novel *in vivo* and *in vitro* model of cutaneous photoaging. J Invest Dermatol 105:269-273, 1995.
3. Bernstein EF, Gasparro FP, Brown DB, Tsunemichi T, Uitto J. 8-methoxypsoralen and ultraviolet A radiation activate the human elastin promoter in transgenic mice: *In vivo* and *in vitro* evidence for gene induction. Photochem Photobiol 64: 369-374, 1996.
4. Bernstein EF, Brown DB, Takeuchi T, Kong SK, Uitto J. Evaluation of sunscreens with various sun protection factors in a new transgenic mouse model of cutaneous photoaging that measures elastin promoter activation. J Am Acad Dermatol 1997;37:725-729.
5. Uitto J, Brown DB, Gasparro FP, Bernstein EF. Molecular aspects of photoaging. Eur J Dermatol 1997;7:210-214.
6. Takeuchi T, Uitto J, Bernstein EF. A novel in vivo model for evaluating agents which protect against ultraviolet A-induced photoaging. J Invest Dermatol 110:343-347, 1998.
7. Bernstein EF, Uitto J, Gasparro F, Brown, DB. The nitroxide tempol affords protection against ultraviolet radiation as assayed using a transgenic mouse model of cutaneous photoaging. Exp Dermatol 10:55-61,2001.
8. Bernstein EF. Reactive Oxygen Species Activates the Human Elastin Promoter in a Transgenic Model of Cutaneous Photoaging. Dermatol Surg 28:132-135, 2002.

I hope this clarifies our submission of data to support our patent application, *Use of serine protease inhibitors in the prevention of photoaging*. If any questions regarding this patent application come up, please do not hesitate to contact me.

Sincerely yours,

Eric F. Bernstein, MD

EFB:jml

Enclosures